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Does intracytoplasmic morphologically selected sperm injection improve embryo development? A randomized sibling-oocyte study

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STUDY QUESTION: Does high-magnification sperm selection influence oocyte fertilization and further embryo development?

SUMMARY ANSWER: The present study did not show a difference in oocyte fertilization rate, nor in embryo development between high-magnification intracytoplasmic morphologically selected sperm injection (IMSI) and conventional ICSI.

WHAT IS KNOWN ALREADY: The presence of nuclear vacuoles in sperm seems to influence embryo development and more specifically blastocyst formation. The use of high magnification for morphological sperm selection prior to ICSI has been associated with higher pregnancy rates and lower miscarriage rates.

STUDY DESIGN, SIZE, DURATION: A prospective sibling-oocyte study was conducted, including 350 ICSI cycles to alleviate male infertility. Cycles were included from March 2010 to November 2011.

PARTICIPANTS/MATERIALS, SETTING, METHODS: On the day of treatment, a high-magnification sperm morphology was assessed on at least 200 spermatozoa. Primary endpoints were oocyte fertilization rate and embryo development. Because embryo transfers were not randomized, the clinical outcome (clinical pregnancy rate per transfer cycle) was descriptive. However, the embryologist selecting the embryos for transfer was blinded for the sperm selection procedure.

MAIN RESULTS AND THE ROLE OF CHANCE: IMSI morphology was assessed in 330 semen samples, resulting in the following distribution: $18.1 \pm 14.8\%$ Grade I, $15.2 \pm 10.3\%$ Grade II, $12.3 \pm 9.1\%$ Grade III and $54.4 \pm 23.2\%$ Grade IV. Oocyte fertilization rate was 79.1 and 77.3% after IMSI and ICSI, respectively (NS, paired *t*-test). Embryo development was similar in both treatment groups up to Day 5 of preimplantation development. Comparable numbers of IMSI-only ($n = 125$) and ICSI-only ($n = 139$) embryo transfers were performed. Clinical pregnancies with fetal heart beat were equally distributed over transfers with embryos from IMSI-only (34.4%) or ICSI-only treatment (36.7%).

LIMITATIONS, REASONS FOR CAUTION: The clinical outcome remains descriptive. No firm conclusions could be drawn on cycle rank as a possible indication for IMSI.

WIDER IMPLICATIONS OF THE FINDINGS: The prevalence of vacuoles in normal-shaped spermatozoa is as low as 27.5%. A routine application of IMSI in unselected artificial reproductive technology patients cannot be advocated.

STUDY FUNDING/COMPETING INTEREST(S): None.

Key words: IMSI / ICSI / sibling-oocyte study / embryo development

Introduction

After the introduction of ICSI in 1992, it became clear that the morphology of the individual spermatozoon used for injection was related to oocyte fertilization and pregnancy outcome (De Vos *et al.*, 2003). Whenever possible, ICSI is performed using morphologically well-shaped spermatozoa selected within the limits of the conventional ICSI inverted microscope magnification of ×400. Bartoov *et al.* (2001) introduced the use of high-power differential interference contrast optics (×1500 optical magnification), allowing the identification and selection of motile spermatozoa with a morphologically normal nucleus and a normal nuclear content. The fine morphology of the sperm nucleus seemed to be positively associated with the pregnancy rate. Since then, the use of high magnification for morphological sperm selection has been associated with higher pregnancy rates, higher delivery rates and lower miscarriage rates in a series of case−control studies, mainly from the same research group (Bartoov *et al.*, 2003; Berkovitz *et al.*, 2005, 2006a,b; Hazout *et al.*, 2006).

At present, however, only few randomized controlled trials are available assessing the advantages of this technique called intracytoplasmic morphologically selected sperm injection (IMSI) over the conventional ICSI procedure (Antinori *et al.*, 2008; Balaban *et al.*, 2011; Wilding *et al.*, 2011). In patients with severe oligo-asthenoteratozoospermia, injection of a maximum of three oocytes per patient (according to the Italian law at that time) by IMSI resulted in a higher clinical pregnancy rate than ICSI, whereas miscarriage rates were not significantly different between both procedures (Antinori *et al.*, 2008). In this study, it was noticed that patients with two or more previously failed ICSI attempts benefited the most from IMSI in terms of pregnancy and miscarriage rates. On the other hand, IMSI did not significantly improve the clinical outcome in an unselected patient population, whereas a subgroup of male-factor infertility patients showed higher implantation rates but not live birth rates with IMSI (Balaban *et al.*, 2011). In contrast, Wilding *et al.* (2011) reported improved clinical results when using IMSI in order to deselect abnormal spermatozoa. It seems obvious that more controlled trials are needed in order to confirm or refute the efficiency of IMSI (Nadalina *et al.*, 2009; Setti *et al.*, 2010). However, at present, the identification of a patient population that might benefit from the IMSI method remains a cumbersome obstacle.

Few studies have investigated the relation between IMSI and embryo development (Vanderzwalmen *et al.*, 2008; Knez *et al.*, 2011; Mauri *et al.*, 2010). Whereas no benefit of the high-magnification sperm selection procedure has been described neither on Day 2 (Mauri *et al.*, 2010) nor on Day 3 (Vanderzwalmen *et al.*, 2008), its positive effect on embryo development may only become overt after the activation of the embryonic genome, i.e. beyond Day 3. Blastocyst formation was indeed affected in a group of 25 patients when morphologically abnormal spermatozoa were used for injection (Vanderzwalmen *et al.*, 2008), although this finding was not confirmed by others. No other study has correlated blastocyst formation to the individual high-magnification morphology of the spermatozoon injected into the oocyte. A comparative study between IMSI and ICSI in a small population presenting with poor-quality sperm and failure to develop blastocysts in a previous ICSI cycle, showed a trend to higher number of blastocysts per cycle in the IMSI group (Knez *et al.*, 2011). However, when blastocysts developed, they did not differ in quality according to the procedure of sperm selection, IMSI or ICSI (Knez *et al.*, 2011).

Because of the scarcity of data on IMSI and embryo development, the present sibling-oocyte study was conducted in order to further evaluate the influence of high-magnification sperm selection on embryo development. In addition to oocyte fertilization and embryo development, all semen samples used for treatment were analysed at high magnification in order to document the prevalence of vacuoles in spermatozoa within a general ICSI population. In the IMSI arm of the study, individual high-magnification morphology of spermatozoa was linked to fertilization and embryo development on Day 3 and 5. Clinical pregnancy with positive fetal heart beat per transfer was the clinical outcome measure. Cycle rank and semen quality were correlated to IMSI and ICSI clinical efficiencies.

Materials and Methods

Study design and patients

A prospective randomized sibling-oocyte study was started in order to compare the effectiveness of sperm selection at high (×1500) magnification (IMSI) and sperm selection after conventional ICSI at ×400 magnification. Effectiveness was defined by fertilization rate and further embryo development, compared on Day 2, 3 and 5. The present study started in March 2010 and ended in November 2011.

The study design involved randomization of oocytes; hence the oocyte was the unit of analysis. Sample size calculation was based on the fertilization rate as the primary outcome, expected to be 75% for ICSI and 81% for IMSI (or a 6% difference). A calculation made to ensure a power of 80% requires 1350 oocytes per group or 2700 oocytes overall, corresponding to ~338 cycles when anticipating a mean of 8 mature oocytes per cycle.

Three hundred and forty couples undergoing ICSI treatment (350 cycles) for a male-factor indication were included. No female factor was involved. The mean rank of attempt was 2.0 ± 1.2 (range: 1–10), but most of the patients were undergoing their first to fourth attempt (Table I). More than 90% of the patients suffered from primary infertility. Patients were not older than 37 years of age and presented with at least 2 and no more than 20 mature oocytes. Cycles with fresh ejaculated spermatozoa that had a concentration ≥0.1 million/ml were included. Frozen

Table I Distribution of cycles according to rank of attempt.

	Total	Primary infertility	Secondary infertility
Rank 1	188	170	18
Rank 2	72[a]	65	7
Rank 3	50[b]	47	3
Rank 4	27[c]	27	0
Rank 5	9	8	1
Rank 6	3	3	0
Rank 10	1	1	0
Total	350	321	29

[a]Including one patient with one previous miscarriage.
[b]Including two patients with one and one patient with two previous miscarriage(s).
[c]Including one patient with one previous miscarriage.

and surgically extracted sperm preparations and embryo-biopsy cycles were excluded from the study.

Fertilization rate and embryo development (stage and quality) were primary outcomes, whereas clinical pregnancy rate per transfer cycle was evaluated as a secondary outcome measure. Additionally, a high-magnification semen morphology was assessed whenever possible (depending on sufficient concentration and/or motility), in order to assess the prevalence of vacuoles in semen samples within an ICSI population. Embryo development up to Day 3 was available for all patients included ($n = 350$), whereas Day 5 blastocyst development was available only for those patients scheduled for Day 5 transfer ($n = 187$).

Ovarian stimulation and oocyte collection

Two ovarian-stimulation protocols were used: the long GnRH-agonist protocol (Suprefact, Hoechst, Frankfurt, Germany) with hMG (Menopur, Ferring Pharmaceuticals A/S; Van de Velde et al., 1998) or the GnRH-antagonist protocol (Orgalutran, MSD, Oss, The Netherlands or Cetrotide, Merck-Serono, Overijse, Belgium) with recFSH (Puregon, MSD or Gonal-F, Merck-Serono; Kolibianakis et al., 2004). Final oocyte maturation was achieved by 5000 or 10 000 IU of hCG (Pregnyl) when at least three follicles of minimum 17 mm diameter were present at ultrasound. Oocyte retrieval was carried out by vaginal ultrasound-guided puncture 36 h after hCG was administered. Intravaginally administered progesterone (Utrogestan, Besins, Brussels, Belgium) was used for luteal phase supplementation.

After puncture and denudation, mature oocytes were randomly assigned to IMSI or ICSI treatment, the cohort being divided into two groups. A randomization list was then used in order to tell which treatment the first half of the oocytes received. In case of an odd number of MII oocytes, the supernumerary oocyte was always assigned to the first half (which then randomized between IMSI and ICSI).

ICSI procedure

Sperm preparation for ICSI was performed as described before (Van de Velde et al., 1997). After denudation, metaphase-II oocytes were immediately injected with a single spermatozoon into the ooplasm, according to Van Landuyt et al. (2005). The injected oocytes were incubated at 37°C (6% CO_2, 5% O_2 and 89% N_2) in 25 μl droplets of Quinn's Advantage™ sequential media (SAGE, cleavage and blastocyst medium, Rochford Medical Ltd, Coventry, England) placed under oil suitable for embryo culture (Ovoil, Vitrolife, Göteborg, Sweden). Embryos were transferred to blastocyst medium on Day 3 of culture.

IMSI procedure and real-time high-magnification semen morphology

For the IMSI procedure, a Nikon inverted microscope equipped with high-resolution Nomarski optics (×100) enhanced by a videozoom and digital imaging was used. No immersion oil was used in order to allow immediate injection of the oocytes. A fraction of the sperm suspension was added to a polyvinylpyrrolidone droplet (Vitrolife) covered with oil (Ovoil) within a glass-bottomed fluorodish (World Precision Instruments, Herts, England). Spermatozoa without vacuoles were selected for injection into the oocytes or otherwise; second-best spermatozoa with the least number of vacuoles and/or other abnormalities were selected for injection. For each cycle included, high-magnification semen morphology was assessed on at least 200 spermatozoa whenever possible, based on the grading system by Vanderzwalmen et al. (2008). Four different grades according to the presence or size of vacuoles were distinguished: Grade I with absence of vacuoles, Grade II with maximum of two small vacuoles, Grade III with more than two small vacuoles or at least one large

vacuole and Grade IV with abnormal head shapes or other abnormalities with or without vacuoles. The latter category can be identified clearly even at low magnification (×400).

Fertilization and embryo development

Sixteen to eighteen hours post-injection, fertilization was assessed and further development of the embryos was evaluated on a daily basis. On Day 2, top-quality embryos were defined as presenting with four equally sized blastomeres and no more than 10% of fragmentation. On Day 3, top-quality embryos had at least seven blastomeres, with volumes proportionally to the division pattern and no more than 10% of fragmentation. Day 5 blastocyst evaluation was done as described by Gardner et al. (2000). Top-quality blastocysts were defined as at least expansion stage 3, an inner cell mass Grade A and a trophectoderm Grade A or B. The total number of blastocysts was defined as the number of embryos cavitating on Day 5.

Clinical outcome

A pregnancy was defined as a rise in serum hCG (>20 IU), measured 14 days after oocyte aspiration, and repeated 3 days later. A clinical pregnancy was defined by the presence of a gestational sac at ultrasound performed at 7 weeks of gestation (Zegers-Hochschild et al., 2006). A biochemical pregnancy involved evidence of conception based only on biochemical data before ultrasound evidence of pregnancy. The definitions for (pre)clinical abortion and ectopic pregnancy were also adopted from Zegers-Hochschild et al. (2006).

Statistical analysis

Fertilization rate and embryo development were analysed by the paired Student's t-test. Clinical outcome was analysed by χ^2 test. Predictive parameters for pregnancy (female age, rank of attempt, semen quality in terms of preliminar Kruger morphology, concentration, progressive motility and IMSI morphology) were compared by the unpaired Student's t-test. All tests were interpreted with a significance level of 95% ($P < 0.05$).

Results

Real-time high-magnification semen morphology

Baseline semen characteristics (concentration and motility) of the semen samples used for the IMSI/ICSI sibling cycles are given in Table II. Eight semen samples had a concentration <0.1 million/ml (range: 0.002–0.06). Kruger morphology was assessed on a diagnostic

Table II Baseline semen characteristics and real-time high-magnification morphology.

Concentration (million/ml)	36.2 ± 54.2
% A + B motility	39.8 ± 22.7
% Normal Kruger morphology	3.7 ± 3.3
% Grade I (normal shape, no vacuoles)	18.1 ± 14.8
% Grade II (normal shape, ≤2 small vacuoles)	15.2 ± 10.3
% Grade III (normal shape, >2 small or ≥1 large vacuoles)	12.3 ± 9.1
% Grade IV (large vacuoles and/or amorphous)	54.4 ± 23.2

Values ± SD.

semen sample preliminar to the treatment cycle. All but 20 semen samples allowed assessment at real-time high-magnification on at least 200 spermatozoa. Because of concentration and/or motility constraints in 18 cycles, 100, and in 7 cycles, 50 spermatozoa were analysed. Grade IV was observed in 54.4% of the spermatozoa, mainly because of being amorphous in shape and/or presenting with large vacuoles. This type of spermatozoa can easily be recognized at ×400 conventional ICSI magnification too. Besides these, 18.1% of the spermatozoa were normal-shaped and without vacuoles (Grade I), while 15.2% displayed less than two small vacuoles (Grade II) and 12.3% more than two small or at least one large vacuole (Grade III).

In the IMSI arm of the study (n = 1557 oocytes, n = 1519 spermatozoa assessed), the majority of oocytes (90.4%) could be injected with Grade I spermatozoa. Only 8.5% of the oocytes were injected with Grade II spermatozoa. The obligatory use of Grade III (n = 6) and Grade IV (n = 10) spermatozoa, because no Grade I or II were available in the sample, was restricted to only 1.1% of the oocytes (or five patients of the cohort). Figure 1 represents the distribution
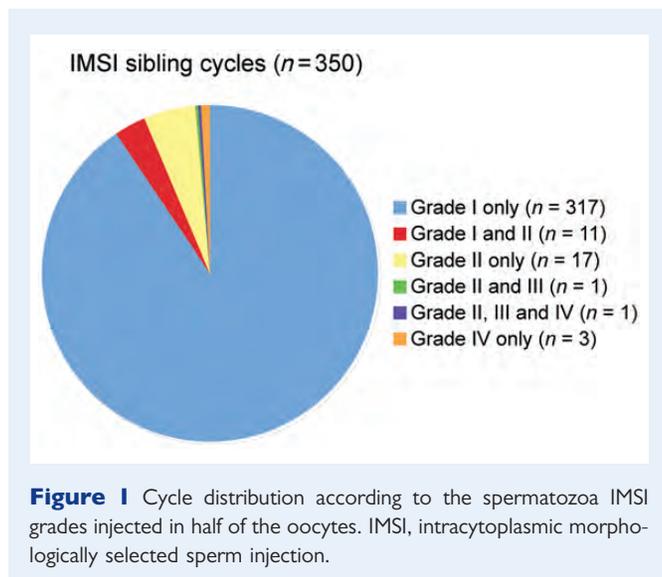
Figure 1 Cycle distribution according to the spermatozoa IMSI grades injected in half of the oocytes. IMSI, intracytoplasmic morphologically selected sperm injection.

of the cycles according to the sperm grade used for IMSI, indicating extremely few cycles where Grade III or IV had to be injected in the absence of higher grade spermatozoa (n = 5). Fertilization rates for the different grades of spermatozoa were 78.9% for Grade I, 67.4% for Grade II and 56.3% for Grade III and IV.

Fertilization and embryo development

Table III shows that the fertilization rate was not different between IMSI and ICSI (79.1 and 77.3% per injected MII oocyte). ICSI resulted in significantly more top-quality embryos on Day 2; however, this difference was neither present on Day 3 nor on Day 5 (total blastocyst formation). Likewise on Day 5, there was no difference in top-quality, good-quality or early blastocyst formation. Similarly, mean developmental stage on Day 2 and 3 was not different between IMSI and ICSI.

Day 5 blastocyst development was available for 187 out of 350 cycles. Total blastocyst formation obtained with Grade I, II, III and IV spermatozoa is represented in Table IV. Too few Grade III and IV spermatozoa were used for oocyte injection in order to give relevant data. However, it seems that blastocyst formation is not excluded when using these grades of spermatozoa (one good-quality blastocyst obtained with each a Grade III and a Grade IV spermatozoon).

Clinical outcome

The clinical outcome is summarized in Table V. Of the 350 cycles started, 337 resulted in embryo transfer. Reasons for no embryo transfer were insufficient embryo quality (either on Day 3, n = 1 or on Day 5, n = 10), failed fertilization (n = 1) or a medical indication (n = 1, hypothyroidism). Apart from the mixed non-informative transfers, about similar numbers of IMSI-only (n = 125) and ICSI-only (n = 139) transfers were performed, confirming the observation of similar embryo quality after IMSI and ICSI.

The mean female age and the rank of attempt were similar in the IMSI and ICSI transfer groups, as was the mean number of embryos replaced (± SD): 1.24 ± 0.43 and 1.23 ± 0.44, respectively. Diminished ovarian reserve (≤5 mature oocytes) was also excluded as a possible confounding factor on the clinical outcome (29 of 125

Table III Fertilization and embryo development.

	IMSI (n = 1557)	ICSI (n = 1548)	P-value, paired t-test
Fertilization (% per injected MII oocyte)	79.1 ± 1.2	77.3 ± 1.3	0.220
Embryo quality			
Day 2 (% top-quality embryos/2-PN)	35.0 ± 1.8	38.5 ± 2.0	0.047
Day 3 (% top-quality embryos/2-PN)	37.0 ± 1.9	38.5 ± 1.9	0.362
Day 5 (% top-quality blastocysts/2-PN)	9.8 ± 1.3	11.4 ± 1.6	0.428
Day 5 (% total blastocyst formation/2-PN)	39.9 ± 2.3	43.4 ± 2.6	0.247
Mean developmental stage			
Day 2	3.85 ± 0.06	3.87 ± 0.05	0.557
Day 3	7.19 ± 0.09	7.31 ± 0.09	0.098

M-II, metaphase-II oocyte; IMSI, intracytoplasmic morphologically selected sperm injection.
Values ± SE.
Total blastocyst formation includes top, good and early blastocysts.

Table IV Total blastocyst formation according to the different grades of spermatozoa for those patients scheduled for Day 5 transfer (n = 187 out of 350 treatment cycles).

	Cycles		MII, n	2-PN, n (%)	Morphological grade			
	n	(%)			I	II	III	IV
Grade I only	168	89.9	820	650 (79.3)	39.1%	–	–	–
Grade I and II[a]	7	3.7	42	34 (81.0)	40.0%	42.1%	–	–
Grade II only	9	4.8	48	34 (70.8)	–	50.0%	–	–
Grade II and III[b]	1	0.5	4	3 (75.0)	–	50.0%	100.0%	–
Grade IV	2	1.1	7	2 (28.6)	–	–	–	50.0%

[a]With Grade I and II spermatozoa 15 of 18 (83.3%) and 19 of 24 (79.2%) MII fertilized, respectively.
[b]With Grade II and III spermatozoa 2 of 3 and 1 of 1 MII fertilized, respectively.

Table V Clinical outcome.

	IMSI	ICSI	Mixed
Number of embryo transfers (ET)	125	139	73
Number of Day 3 transfers (%)	47 (37.6)	57 (41.0)	56 (76.7)
Number of Day 5 transfers (%)	78 (62.4)	82 (59.0)	17 (23.3)
Mean female age	30.7 ± 3.5	30.9 ± 3.3	32.1 ± 3.1
Mean rank of attempt	1.7 ± 1.1	1.7 ± 1.2	2.7 ± 1.3
Mean number of embryos replaced	1.2 ± 0.4	1.2 ± 0.4	2.0 ± 0.1
Number of positive hCG (% per ET)	55 (44.0)[a]	68 (48.9)[a]	38 (52.1)
Biochemical pregnancy	5	13	3
Preclinical abortion	4	3	2
Ectopic pregnancy	3	1	1
Clinical pregnancy[b] (% per ET)	43 (34.4)[a]	51 (36.7)[a]	32 (43.8)
Implantation rate per embryo transferred (%)	30.3	32.2	28.6
Delivery follow-up			
Lost follow-up	3	5	4
Elective termination	0	1	2
Clinical abortion	3	1	1
Delivery stillborn	0	2[c]	1[d]
Delivery live born (singleton/twin)	37 (33/4)	42 (40/2)	24 (17/7)

Mean values ± SD.
IMSI, intracytoplasmic morphologically selected sperm injection.
[a]Not significant, χ^2 test.
[b]Clinical pregnancy rate with fetal heart beat at 7 weeks ultrasound.
[c]One twin and one sibling of a twin.
[d]One triplet (despite the transfer of two embryos).

transfers or 23.2% in the IMSI arm and 24 of 139 transfers or 17.3% in the ICSI arm, not different by χ^2 test). In the mixed transfer group, patients were on average 1 year older and had a higher rank of attempt, justifying the replacement of two embryos in that group. In the IMSI transfer group, 38% were Day 3 transfers and 62% were Day 5 transfers. This was similar for the ICSI transfer group (41%

Day 3 transfers and 59% Day 5 transfers). The mixed transfer group showed a higher proportion of Day 3 transfers (77%).

The overall rate of positive hCG per embryo transfer was 44.0% (55 of 125) in the IMSI transfer group and 48.9% (68 of 139) in the ICSI transfer group (NS, χ^2 test, Table V). The clinical pregnancy rate per embryo transfer was not different between the two transfer groups (34.4 and 36.7%). The implantation rate per embryo transferred was similar for IMSI and ICSI. The delivery follow-up is also included in Table V.

Within the IMSI transfer group, no embryos resulting from Grade III and IV spermatozoa were selected for transfer. In 118 IMSI transfer cycles with exclusively embryos resulting from Grade I injection (n = 147), the implantation rate per embryo transferred was 27.2%. Seven transfer cycles involved exclusively embryos resulting from Grade II injection (n = 8). Four implantations with fetal heartbeat were obtained in the latter group.

Predictive parameters for pregnancy and possible indications for IMSI

In the present cohort, clinically pregnant and non-pregnant patients did not differ significantly in female age (30.8 ± 3.5 and 31.3 ± 3.3, $P = 0.0950$), rank of attempt (1.8 ± 1.2 and 1.9 ± 1.3, $P = 0.1576$) or any of the semen characteristics, being preliminary Kruger morphology (3.4 ± 3.4 and 3.9 ± 3.2% normal forms, $P = 0.1624$), sperm count (33.1 ± 36.0 and 37.7 ± 61.6 million/ml, $P = 0.2248$), progressive motility (37.5 ± 22.3 and 41.0 ± 22.8% A + B motility, $P = 0.0848$) or real-time IMSI morphology (16.7 and 18.8% Grade I, $P = 0.1178$; 14.6 and 15.5% Grade II, $P = 0.2109$; 12.4 and 12.2% Grade III, $P = 0.4270$; 56.3 and 53.5% Grade IV, $P = 0.1492$).

Figure 2 represents the patients of the present cohort in their first (n = 151), second (n = 50), third (n = 25) or higher rank of attempt (n = 19) for their first child, having either an IMSI-only or an ICSI-only transfer. Patients that had previous cycles elsewhere before coming to our centre were not taken into account here. The majority of patients were in their first attempt. For these patients, no benefit from IMSI was observed (29.2% clinical pregnancies per IMSI-only transfer compared with 45.6% clinical pregnancies per ICSI-only transfer). Higher rank attempts obviously suffer from small numbers. Nevertheless, IMSI-only transfers showed a tendency towards a higher clinical outcome than ICSI-only transfers, especially for third-rank attempts (not significant).
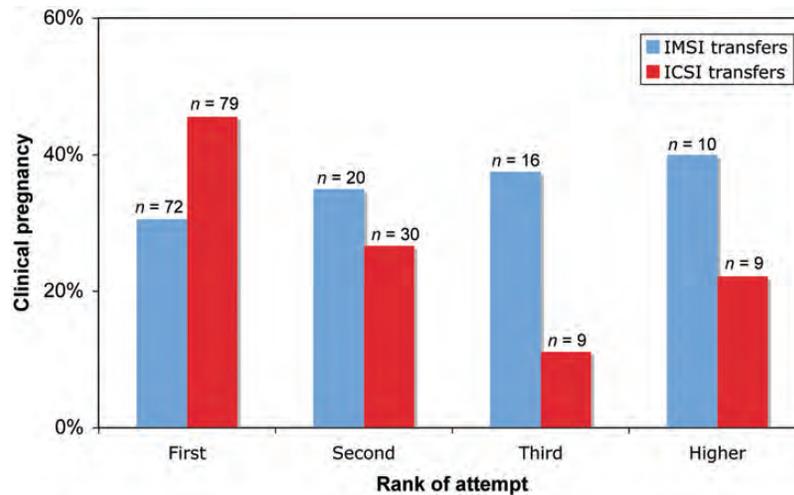
Figure 2 Relationship between rank of attempt and clinical pregnancy rates for IMSI-only and ICSI-only transfers. IMSI, intracytoplasmic morphologically selected sperm injection.
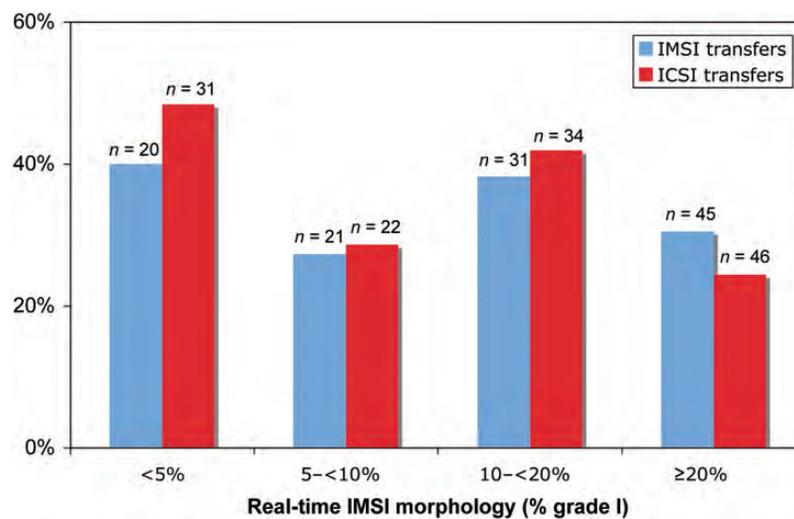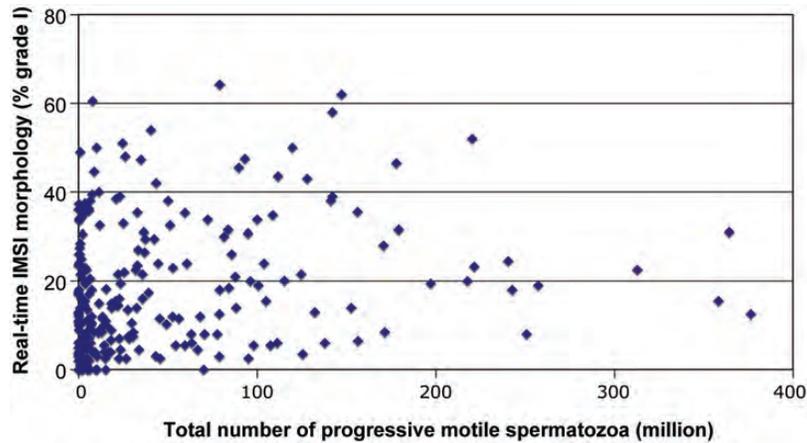

Figure 3 Relationship between real-time IMSI morphology (% Grade I) and clinical pregnancy rates for IMSI-only and ICSI-only transfers. IMSI, intracytoplasmic morphologically selected sperm injection.

In Fig. 3, clinical pregnancy rates for IMSI-only and ICSI-only transfers were related to the sperm quality in terms of real-time IMSI morphology (% Grade I). Clinical pregnancies can be obtained with semen samples showing <5% Grade I spermatozoa, even with conventional ICSI. Each semen morphology category showed similar clinical results with IMSI-only or ICSI-only transfers. Figure 4 shows that there is no correlation between real-time IMSI morphology (% Grade I) and total number of progressive motile spermatozoa (million) present in the semen sample. Each category of total number of progressive motile spermatozoa showed similar clinical outcomes with IMSI-only and ICSI-only (Fig. 5).

Discussion

The present sibling-oocyte study compares conventional ICSI with a sperm selection method using higher magnification (IMSI). Any difference neither in oocyte fertilization rate, nor in embryo quality was observed. Also, the clinical pregnancy rate and the implantation rate per embryo transferred were similar for IMSI-only and ICSI-only transfers.

The present data do not support any benefit of IMSI in a non-selected population as tested here, with fresh ejaculated sperm containing ≥ I million/ml. The female partners were not older than 37 years of age, were undergoing their first to fourth treatment cycle and presented

Figure 4 Relationship between total number of progressive motile spermatozoa (million) and real-time IMSI morphology (% Grade I). IMSI, intra-cytoplasmic morphologically selected sperm injection.
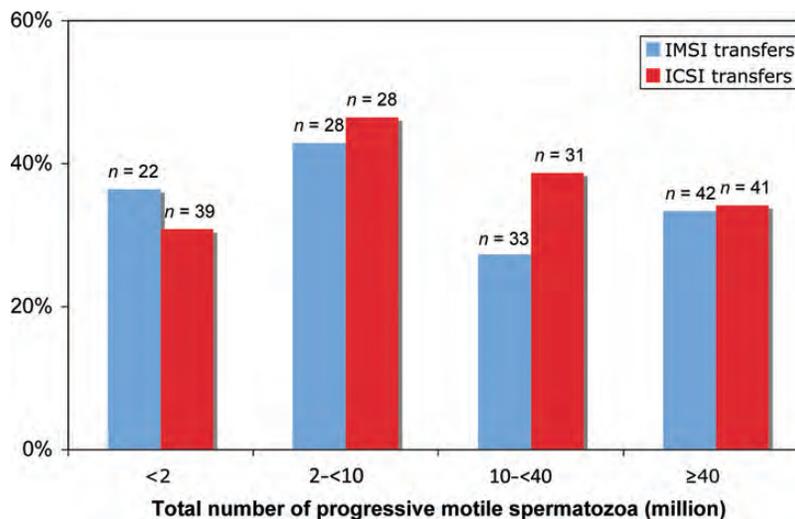
Figure 5 Relationship between total number of progressive motile spermatozoa (million) and clinical pregnancy rates for IMSI-only and ICSI-only transfers. IMSI, intracytoplasmic morphologically selected sperm injection.

no infertility factor. All couples were enrolled for ICSI because of oligo-astheno-teratozoospermia. The present ICSI patient population is thus comparable with the one studied by Balaban et al. (2011), referred to as an unselected male-infertility population. Antinori et al. (2008), using similar poor-morphology semen samples, however, did not report the concentration or motility of their semen samples, and the patient population studied by Wilding et al. (2011) was neither well defined in terms of semen characteristics (samples between 1 and 20 million/ml).

Preliminary semen morphology according to Kruger's criteria was not correlated with real-time IMSI morphology in this study (Oliveira et al., 2009). The aim was to describe the prevalence of vacuoles within a given sperm sample and within a given ICSI patient population. Earlier studies on IMSI did not report these frequencies, with one

exception reporting 33–35% spermatozoa with a vacuolar nucleus (Balaban et al., 2011). This percentage corresponds well with our findings (27.5% Grade II and Grade III spermatozoa). Our results showed that the majority of spermatozoa (54.4%) were amorphous in head shape, easily recognizable in conventional ($\times 400$) ICSI as well. We reported a prevalence of normal spermatozoa without vacuoles as high as 18%, which means that about one in five spermatozoa passing through the microscopic field is suitable for microinjection into the oocytes. More recent studies on real-time IMSI morphology reported lower percentages of normal spermatozoa without vacuoles: 1.5–1.8% (Oliveira et al., 2010a,b, 2011). A possible explanation for this discrepancy might be the use of immersion oil, yielding a much higher resolution than without immersion oil as in the present study,

which was a practical consideration for consecutive microinjection. The presence of 25–26% of spermatozoa with large nuclear vacuoles (Oliveira et al., 2010a) seems better correlated with our findings (12% Grade III spermatozoa). When selecting spermatozoa with conventional ICSI, Wilding et al. (2011) reported 12.1% of them showing multiple vacuoles and 20.8% presenting with vacuoles over 4% of the area when assessed at high magnification under immersion oil. Taken together, this prevalence is higher than our finding (13% Grade III spermatozoa), however, not readily comparable because of preselection at ×400, whereas our prevalence involved the semen sample without preselection at ×400. It seems justified to estimate the prevalence of vacuoles in normally shaped sperm heads (Watanabe et al., 2011), showing that those without vacuoles on the one hand and those with large vacuoles on the other hand are very rare in patients (respectively, 2.6 and 4.6%). The prevalence of small vacuoles found in normally shaped spermatozoa was extremely high (92.8% in patients, comparable with 95.8% in fertile donors), making the authors conclude that these should be considered as a common feature in normal human sperm and not associated with pathology or DNA damage.

In the present study, the presence of certain vacuoles might have been overlooked due to resolution constraints. Whereas the origin of large vacuoles has been studied (Kacem et al., 2010; Boitrelle et al., 2011), the nature of small vacuoles remains unclear. Moreover, the question arises what the significance of these small vacuoles in spermatozoa may be in terms of oocyte fertilization or further embryo development up until Day 5. Although fertilization with Grade II spermatozoa (67.4%) was lower than with Grade I spermatozoa (78.9%), the present study does not show a difference in total blastocyst formation once the oocyte is fertilized. This finding for Grade II spermatozoa corresponds with Vanderzwalmen et al. (2008). In our study group, too few Grade III and IV spermatozoa were used for microinjection in order to draw a valid conclusion regarding blastocyst formation. While Vanderzwalmen et al. (2008) found that blastocyst formation is severely affected by the presence of large vacuoles and/or abnormal head shapes, the present study only shows that blastocyst formation was not jeopardized when using either Grade III or IV spermatozoa. Beyond blastocyst formation, the implantation rate per embryo transferred was not affected when the embryos were derived from Grade II spermatozoa. Grade III- and IV-derived embryos were not selected for transfer.

The present study was sibling-oocyte comparison with oocyte fertilization rate and embryo development as primary endpoints. No significant differences were observed between conventional ICSI and IMSI for these endpoints. In the literature, all publications on IMSI are in agreement that there is no difference between IMSI and ICSI for fertilization rates. Most studies also agree on comparable embryo development between IMSI and ICSI on Day 2 (Mauri et al., 2010; Oliveira et al., 2011) and Day 3 (Vanderzwalmen et al., 2008; Balaban et al., 2011; de Almeida Ferreira Braga et al., 2011; Knez et al., 2011). In contrast, Wilding et al. (2011) reported improved embryo development with IMSI (37.2% Grade I embryos) when compared with ICSI (26.8% Grade I embryos). For Day 5 blastocyst formation following the injection of vacuolated spermatozoa, data remain limited (Vanderzwalmen et al., 2008; 25 patients, 143 zygotes).

In order to draw firm conclusions regarding the benefit of IMSI within the artificial reproductive technology (ART) setting, randomized control trials are needed in selected subgroups. Three such studies are available in the literature to date. IMSI significantly improved clinical pregnancy rate when compared with conventional ICSI (39.2 versus 26.5%) within a population of severe male-factor infertility, without affecting the miscarriage rate (Antinori et al., 2008). But this finding was not confirmed by Balaban et al. (2011) in an infertile population with similar poor semen morphology, although with a higher mean sperm count and motility (clinical pregnancy rate 54.0% for IMSI and 44.4% for ICSI, not significant). Wilding et al. (2011) reported improved embryo morphology together with significantly increased pregnancy (respectively, 65.6 and 40.0%) and implantation rates (respectively, 24.2 and 14.8%) when using IMSI for couples with 1–3 years of infertility and semen characteristics between 1 and 20 million/ml. The present cohort of single embryo transfers in a comparable patient population does not support an improved clinical outcome with IMSI compared with ICSI. Therefore, a routine application of IMSI in unselected ART patients cannot be advocated.

Moreover, it remains unclear to date which target population might benefit from the more precise sperm selection method, either couples with a history of failed ICSI procedures (Antinori et al., 2008) or severe male-factor infertility patients (Balaban et al., 2011). The present study included too few higher rank cycles in order to allow firm conclusions in this respect. However, patients in their first treatment cycle did not benefit from an IMSI-only transfer. With respect to male-factor infertility as a potential target group for IMSI, a correlation between real-time IMSI morphology and clinical outcome was analysed. Whereas Bartoov et al. (2002) reported no pregnancies below a threshold of 20% spermatozoa with a morphologically normal nucleus, our study contradicts this finding. Even with <5% Grade I spermatozoa in the semen sample, acceptable clinical pregnancy rates were obtained, noteworthy, with both IMSI and conventional ICSI. None of the four real-time IMSI semen morphology categories showed a better clinical result with IMSI than with conventional ICSI, in contradiction to the report by Wilding et al. (2011), showing a specific benefit of IMSI for lower morphology categories. Our finding does not support a prior screening of semen samples for the presence of vacuoles in order to recommend ICSI or IMSI. Similarly, a possible benefit with IMSI was neither correlated to the total number of progressive motile spermatozoa present in the semen sample.

In conclusion, the proportion of spermatozoa with vacuoles within semen samples hardly compromised the selection of suitable spermatozoa for oocyte injection. The use of so-called 'second-best' spermatozoa had no major implications on fertilization and blastocyst formation. Within the present sibling-oocyte design, IMSI and conventional ICSI were comparable in terms of oocyte fertilization rate and embryo development up to the blastocyst stage. Moreover, the clinical outcome was similar for IMSI-only and ICSI-only transfers. Whereas higher rank attempts might benefit from IMSI, the efficiency of IMSI in moderate male-factor infertility, allowing the application of IMSI, seems very doubtful.

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(equipped with high-resolution Nomarski optics enhanced by a video-zoom and digital imaging) on demonstration for the period of the study.

Authors' roles

A.D.V, H.V.D.V., H.T. and G.V. substantially contributed to the study conception and design as well as to the analysis and interpretation of the data. Acquisition of data was done by A.D.V., G.B., G.E., N.F., G.M., S.T. and A.V.; A.D.V. drafted the article. All authors revised it critically and finally approved the version to be published.

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Conflict of interest

None declared.

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